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Neutrophil recruitment is inhibited by nicotinamide in experimental pleurisy in mice

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ABSTRACT

Several emerging lines of evidence support an anti-inflammatory role for nicotinamide and other vitamin B components. However, the mechanisms underlying their activity remain unclear. In the present study, we investigated the ability of nicotinamide to inhibit both neutrophil recruitment in IL-8-, LTB_4- or carrageenan-induced pleurisy in mice and the rolling and adherence of neutrophils. Nicotinamide inhibited IL-8-, LTB_4- and carrageenan-induced neutrophil migration, KC production and carrageenan-induced neutrophil rolling and adherence. We propose that the effects of nicotinamide in inhibiting neutrophil recruitment in carrageenan-induced pleurisy may be due to the ability of nicotinamide to inhibit the action of IL-8 and LTB_4, decrease KC production, and inhibit early events that regulate leukocyte migration from blood vessels into tissue.

1. Introduction

Neutrophils are circulating cells that are recruited from blood vessels into inflamed tissues in response to chemoattractants released at the site of injury. Studies of chemoattractant agents show a major role for the CXC chemokines CXCL-8/interleukin (IL-)8, keratinocyte-derived chemokine (KC/CXCL1, a mouse homologue of human IL-8), and lipopolysaccharide-induced CXC chemokine (LIX/CXCL5) and lipid mediators such as leukotriene (LT)B_4, which act directly on the surface of neutrophils to induce recruitment *in vivo* (Baggiolini et al., 1989; Kobayashi, 2008; Samuelsson, 1983; Smith et al., 2008).

At the site of injury, neutrophil accumulation plays an important role in tissue damage by releasing proteases contained within their azurophilic granules, such as neutrophil elastase, cathepsin G and proteinase-3 (Faurschou and Borregaard, 2003; Korkmaz et al., 2010), lysosomal enzymes, and reactive oxygen species that in turn promote lesions in surrounding tissues and contribute to the severity of inflammatory disease (Kobayashi and DeLeo, 2009; Weiss, 1989). Thus, a better understanding of the pathways and chemoattractant molecules required for neutrophil migration from the blood vessels to the inflamed tissues may aid in the development of new therapeutic strategies for the treatment of inflammatory diseases.

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that transfers adenosine diphosphate-ribose units from nicotinamide adenine dinucleotide (NAD^+) to nuclear proteins, thereby regulating gene expression, DNA repair and apoptosis (Ziegler, 2000). There are several lines of evidence supporting a role for PARP-1 activation in the development of an inflammatory response (Hassa and Hottiger, 2002; Szabo et al., 1997). PARP-1 regulates the function of several transcription factors, including enhancing nuclear factor kappa B (NF-κB)-mediated transcription, and NF-κB is a cellular pro-inflammatory protein implicated in the expression of inflammatory mediators (Virag and Szabo, 2002). The inflammatory role of PARP-1 is further supported by studies from Oliver et al. (1999) demonstrating that PARP-1-deficient mice exhibit reduced expression of NF-κB and other inflammatory mediators, including nitric oxide, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, and reduced cytokine production (Oliver et al., 1999).

Nicotinamide, a component of vitamin B_3, is the amide of nicotinic acid, a PARP-1 inhibitor and a precursor of NAD^+, and thus controls the cellular levels of PARP-1 and consequently the cellular alterations induced by PARP-1, including those related to establishing inflammation resulting from the excessive activation of this enzyme (PARP-1). Thus, it has been suggested that nicotinamide may contribute to the control of inflammatory diseases (Uchigata et al., 1982). Although there is evidence supporting a role for nicotinamide in the control of inflammation, its role in neutrophil recruitment and the mechanisms underlying its involvement in neutrophil recruitment as well as its relevance to the modulation of microvascular events leading

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to neutrophil migration from blood vessels into inflamed tissue remain to be elucidated. In the present study, we evaluated the effects of nicotinamide in carrageenan-induced neutrophil recruitment, neutrophil rolling and adherence.

2. Materials and methods

2.1. Reagents

Nicotinamide, carrageenan, zymosan and carboxymethylcellulose were purchased from Sigma (St. Louis, MO). Nicotinamide was prepared in a 0.5% carboxymethylcellulose sodium salt suspension in saline immediately before the experiments, and the volume of *per os* (p.o.) administration of nicotinamide was 8 ml/kg. Leukotriene B_4 (LTB_4) was purchased from Cayman Chemical (Ann Arbor, MI, USA), and recombinant human IL-8 and murine KC ELISA kit were obtained from Peprotech (Rock Hill, NJ, USA). Carrageenan, IL-8, zymosan and LTB_4 were dissolved in PBS (pH 7.4). IL-8 was dissolved in PBS containing 0.01% bovine serum albumin (Sigma). LTB_4 and IL-8 were dissolved as 1 mg/ml final stock solutions and stored at −20 °C until use. The endotoxin level in the IL-8 solution was <0.1 ng/μg protein (maximal injection into the peritoneal cavity of contaminating LPS was 0.01 ng) and was much lower than the dose of endotoxin needed to induce neutrophil migration in the model (>50 ng/cavity). Control mice received drug vehicle.

2.2. Animals

Male BALB/c mice (18–22 g) were used throughout these experiments. Animals were housed in a temperature-controlled room with free access to food and water. Throughout the experiments, the animals were managed in accordance with the principles and guidelines for the care of laboratory animals, and the experimental procedures were previously approved by the Ethics Committee on Animal Experimentation (CETEA, protocol no. 24/2011) of the Federal University of Minas Gerais (UFMG).

2.3. Carrageenan-, zymosan- or neutrophil chemoattractant-induced leukocyte migration into the pleural cavity

Carrageenan (200 μg/0.1 ml/cavity), zymosan (200 μg/0.1 ml/cavity), IL-8 (60 ng/0.1 ml/cavity), LTB_4 (500 ng/0.1 ml/cavity) or vehicle (PBS/0.1 ml/cavity) were injected intrapleurally (i.pl.) into mice, which were sacrificed in a CO_2 chamber 4 h after the i.pl. injection. The cells present in the cavity were harvested by injecting 2 ml PBS, and total cell counts were performed in a modified Neubauer chamber using Turk's stain (Klein et al., 2001). Differential cell counts were performed on cytospin preparations, which were stained with May–Grunwald and Giemsa to identify cell types according to standard morphologic criteria. The results are presented as the number of cells per cavity. The dose of LTB_4 used in this study was previously shown to effectively induce *in vivo* leukocyte recruitment in the same pleurisy model (Klein et al., 2001), and the IL-8 dose used was based on a previous study of *in vivo* neutrophil recruitment (Ramos et al., 2003).

2.4. Effects of nicotinamide treatment on carrageenan-, zymosan-, IL-8- or LTB_4-induced neutrophil recruitment

To evaluate the effects of nicotinamide treatment on the neutrophil recruitment induced by carrageenan (200 μg/cavity), zymosan (200 μg/cavity), IL-8 (60 ng/cavity) or LTB_4 (500 ng/cavity), mice were treated p.o. with nicotinamide (250, 500 or 1000 mg/kg) or with vehicle (carboxymethylcellulose) twice, 0.5 h before and 1 h after the i.pl. injection of inflammatory stimuli. In some experiments, mice were post-treated with nicotinamide (250, 500 or 1000 mg/kg) 2 h after the carrageenan (200 μg/cavity) i.pl. injection. The number of infiltrating neutrophils was assessed 4 h after the injection of inflammatory stimuli.

2.5. Effects of nicotinamide on carrageenan-induced neutrophil recruitment in intravital studies of cremaster muscle microcirculation

To study the effects of nicotinamide on the behavior of leukocytes in the microcirculation and adjacent connective tissue, we performed intravital studies in a mouse cremaster muscle preparation, as described previously (Kubes et al., 1991). The mouse cremaster muscle preparation is a very well-established preparation that has been used to study the skeletal muscle microcirculation in intravital studies (Hickey et al., 1997; Kunkel et al., 1996; Vicaut and Stucker, 1990), allowing the visualization and quantification in real time of rolling leukocytes and adherent leukocytes to the vascular endothelium.

Mice were gently restricted and treated with nicotinamide (1000 mg/kg) p.o. 0.5 h before and 1 h after the injection of carrageenan (100 μg/0.1 ml) or PBS on the left side of the scrotal tissues with a 30 G needle, as described previously (Kubes et al., 1991). Four hours after the carrageenan injection, mice were anesthetized by i.p. injection of a mixture of 10 mg/kg xylazine and 200 mg/kg ketamine hydrochloride, and the cremaster muscle was carefully dissected and exposed surgically for intravital microscopy studies as previously described (Menezes et al., 2008). A fluorescence microscope (Olympus) with a 10× objective and a 10× eyepiece was used to examine the microcirculation in the cremaster. A video camera (Sony, Japan) was used to project the images onto a monitor, and the images were recorded for playback analysis using a DVD recorder. Single unbranched cremaster venules (25–40 μm in diameter) were selected, and to minimize variability, the same section of cremaster venule was observed throughout the experiment. Three 1-min films were recorded, and the results were expressed as the average of the analysis of the three films. The number of rolling and adherent leukocytes was determined offline during the analysis of video playback.

Rolling leukocytes were defined as those cells moving at a velocity less than that of the erythrocytes within a given vessel. The flux of rolling cells was determined as the number of rolling leukocytes passing a given point in the venule per minute, and the rolling leukocyte results were expressed as cells/min. To evaluate leukocyte adhesion, leukocytes were considered adherent when they remained stationary for at least 30 s. The total leukocyte adhesion was quantified as the number of adherent cells observed within a 100-μm length of venule in a total time of 1 min and was expressed as cells/100 μm.

2.6. Histology

At the end of each intravital microscopy experiment, cremaster muscles were removed and fixed in 10% neutral buffered formalin. The tissues were dehydrated in an ethanol series, embedded in paraffin, cut into 4-μm sections, stained with hematoxylin and eosin and examined under direct light microscopy.

2.7. Measurement of KC

Frozen supernatants obtained from pleural cavity washes at different times after carrageenan or PBS challenges were used to detect KC. For these experiments, the pleural cavities of mice treated with nicotinamide (1000 mg/kg) 0.5 h before i.pl. carrageenan injection (200 μg/cavity) and 0.5 h after carrageenan injection were washed with 1.0 ml ice-cold PBS, then immediately frozen and stored at −70 °C until the assay was performed. The presence of KC was determined with a specific KC ELISA detection kit (Peprotech, Rock Hill, NJ, USA) according to the instructions of the manufacturer.

2.8. Statistical analysis

All results are presented as the mean ± standard error of the mean (S.E.M.). The results were analyzed with GraphPad Prism® 5.0 software (San Diego, CA, USA). Groups were compared by one-way ANOVA, and differences between groups were assessed with the Bonferroni post-test; $P<0.05$ was considered to be significant.

3. Results

3.1. Carrageenan- or zymosan-induced neutrophil recruitment in the mouse pleural cavity is inhibited by nicotinamide

At a dose of 1000 mg/kg, the double nicotinamide treatment (p.o. 0.5 h before and 1 h after the i.pl. injection of carrageenan or zymosan) inhibited neutrophil recruitment 4 h after challenge (Fig. 1A). As shown in Fig. 1B, the double nicotinamide treatment inhibited zymosan-induced neutrophil recruitment at all nicotinamide doses. Interestingly, p.o. administration of nicotinamide 2 h after carrageenan i.pl. injection failed to inhibit the carrageenan-induced neutrophil recruitment observed at 4 h after challenge (Fig. 1C). The number of mononuclear cells in the pleural cavity of carrageenan-treated mice was not affected by the double nicotinamide treatment (Table 1).

3.2. Nicotinamide inhibits LTB_4- or IL-8-induced neutrophil recruitment and KC production in the mouse pleural cavity

Because LTB_4 is a powerful chemoattractant that is released at the site of inflammation in response to inflammatory stimuli, including carrageenan (Mathieu et al., 1990), we investigated the ability of the double p.o. nicotinamide treatment to inhibit LTB_4-induced neutrophil recruitment in the pleurisy model. The double p.o. nicotinamide treatment significantly suppressed (by 87.5%) the recruitment of neutrophils to the pleural cavities of mice at 4 h after the administration of LTB_4 (Fig. 2A).

LTB_4 appears to mediate the recruitment of neutrophils by acting directly on receptors present on the neutrophil surface (Goldman and Goetzl, 1982) and through the production of the CXC chemokine KC/IL-8. Therefore, we examined whether the double p.o. nicotinamide treatment played a role in inhibiting IL-8-induced neutrophil recruitment and whether nicotinamide could inhibit the release of KC in carrageenan-induced pleurisy. As shown in Fig. 2B, IL-8-induced neutrophil recruitment was significantly inhibited by the double p.o. nicotinamide treatment. Indeed, KC was detected at an early stage (0.5 h) in the supernatants obtained from the pleural cavities of carrageenan-injected mice, and the double p.o. nicotinamide treatment inhibited KC production at 0.5 h (Fig. 3A, carboxymethylcellulose + PBS: 0.09 ± 0.035 ng/ml; carboxymethylcellulose + carrageenan: 0.5 ± 0.035 ng/ml; nicotinamide + carrageenan: 0.2 ± 0.043 ng/ml). Double p.o. nicotinamide treatment failed to inhibit KC production 2 h after the i.pl. carrageenan injection (Fig. 3B, carboxymethylcellulose + PBS: 0.2 ± 0.09 ng/ml; carboxymethylcellulose + carrageenan: 1.0 ± 0.15 ng/ml; nicotinamide + carrageenan: 0.6 ± 0.08 ng/ml).

3.3. Nicotinamide abolished the number of rolling and adhering neutrophils on the vessel wall

To assess and separately study the effects of double nicotinamide treatment on leukocytes in the microcirculation, a cremaster preparation was visualized by fluorescence microscopy. Histological analysis of the microvasculature in the cremaster muscles revealed that there are very few leukocytes surrounding the blood vessel in PBS-injected mice (Fig. 4A), while the number of leukocytes surrounding the vessels increases after carrageenan injection (Fig. 4B). By contrast, the number of leukocytes in the microcirculation was inhibited after double p.o. treatment of mice with nicotinamide (Fig. 4C). The number of adherent leukocytes and rolling cells in the microcirculation in the cremaster muscle preparation was reduced following nicotinamide treatment in carrageenan-treated mice (Fig. 5). These

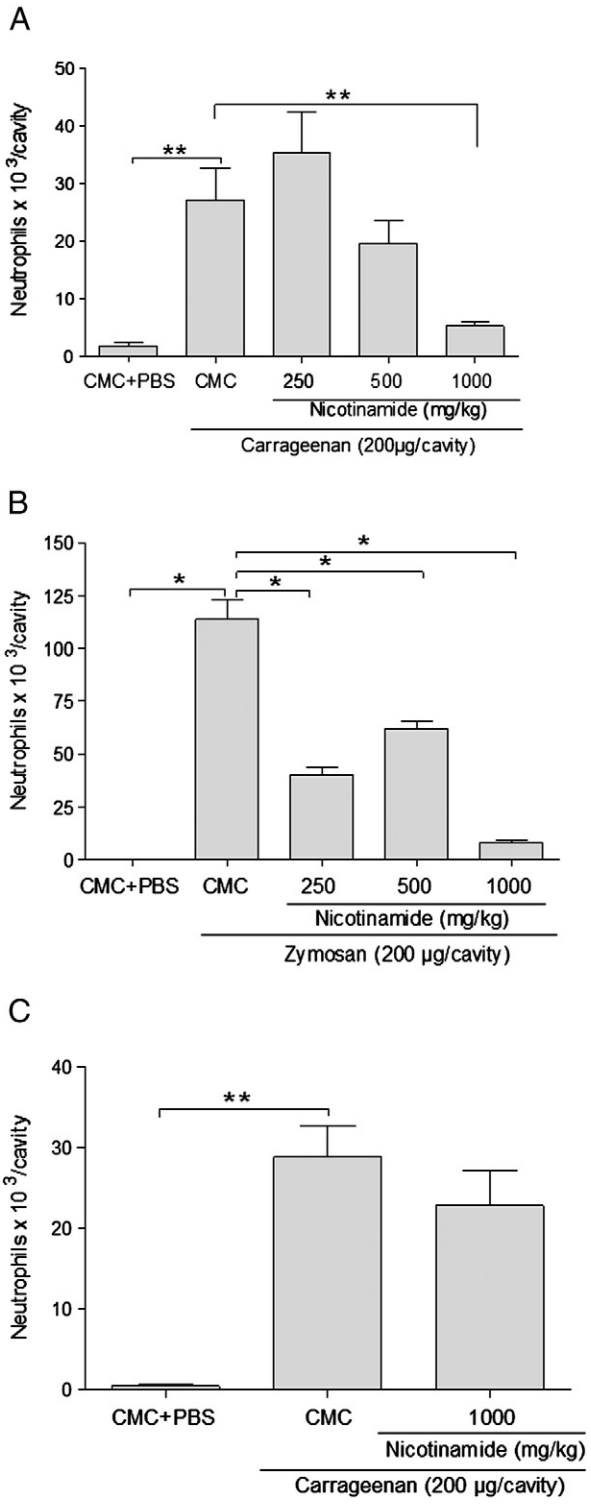

Fig. 1. Double p.o. nicotinamide treatment inhibits carrageenan or zymosan-induced neutrophil recruitment in the mouse pleural cavity.Mice were treated p.o. with different doses of nicotinamide (250–1000 mg/kg) 0.5 h before and 1 h after the i.pl. injection of carrageenan (200 µg/cavity) (A) or zymosan (200 µg/cavity) (B) or were post-treated 2 h after the carrageenan i.pl. injection (C). The number of infiltrating neutrophils was assessed after 4 h. CMC + PBS indicates the carboxymethylcellulose/PBS-treated group. The results are expressed as the mean ± S.E.M. of 5–6 mice/group. *$P<0.001$, **$P<0.01$.

Table 1
Total and differential cell counts ($\times 10^3$/cavity) in response to i.pl. injection of carrageenan in mice treated with different doses of nicotinamide.

	CMC + PBS	Carrageenan 200 μg /cavity			
		CMC	Nicotinamide (mg/kg)		
			250	500	1000
Total	7.7 ± 2.0	33.7 ± 6.0[a]	45.4 ± 9.3	26.0 ± 4.4	11.1 ± 0.5
Neutrophils	1.9 ± 0.6	27.1 ± 5.6[b]	35.3 ± 7.3	19.7 ± 3.9	5.4 ± 0.6[c]
Macrophages	5.7 ± 1.5	6.5 ± 0.8	10.0 ± 2.1	6.3 ± 0.6	5.6 ± 0.9

The results are expressed as the mean ± S.E.M. of 5 mice/group.
[a] $P<0.05$ when compared to CMC + PBS-treated group.
[b] $P<0.001$ when compared to CMC + PBS-treated group.
[c] $P<0.001$ when compared to CMC + carrageenan-treated group. CMC + PBS indicates the carboxymethylcellulose/PBS-treated group.

results are in agreement with the effects of nicotinamide treatment on neutrophil migration in carrageenan-induced pleurisy.

4. Discussion

The role of neutrophils in the pathophysiology of inflammatory diseases is well established, with many lines of evidence supporting a direct correlation between the presence of neutrophils at the inflamed tissue and the progression of inflammatory diseases (Faurschou and Borregaard, 2003; Kobayashi and DeLeo, 2009; Weiss, 1989). It is thus hypothesized that drugs that inhibit neutrophil recruitment and/or activation may become important in new therapeutic strategies for the treatment of inflammatory diseases.

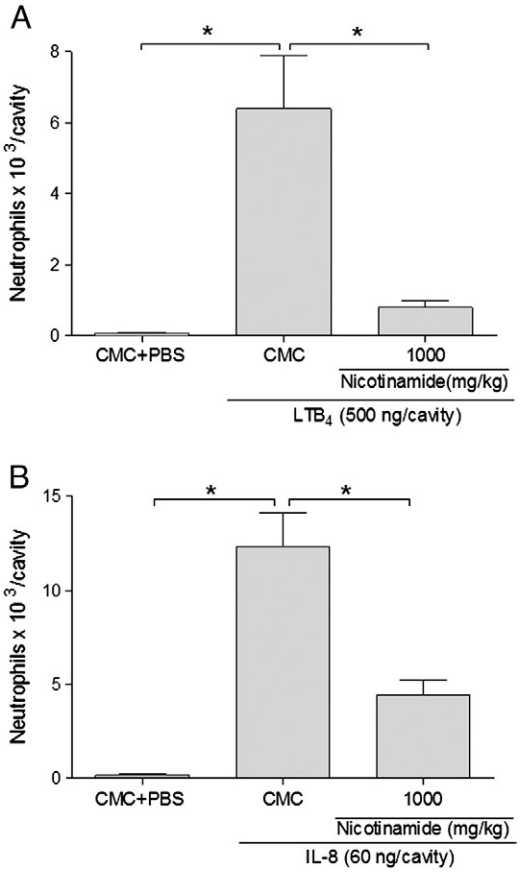

Fig. 2. Effects of nicotinamide double p.o. treatment in response to LTB_4 or IL-8 injection into the mouse pleural cavity.Mice were treated p.o. with nicotinamide (1000 mg/kg) 0.5 h before and 1 h after the i.pl. injection of LTB_4 (500 ng/cavity) (A) or IL-8 (60 ng/cavity) (B), and the number of infiltrating neutrophils was assessed after 4 h. CMC + PBS indicates the carboxymethylcellulose/PBS-treated group. The results are expressed as the mean ± S.E.M. of 6–10 mice/group; *$P<0.001$.

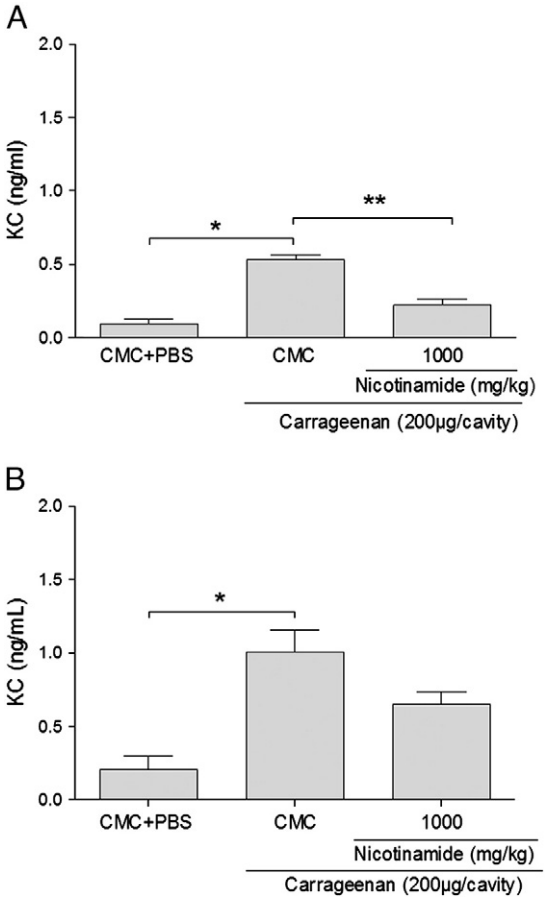

Fig. 3. KC production is inhibited in the pleural cavity following the injection of carrageenan in double *per os*-treated mice.Mice were treated p.o. with nicotinamide (1000 mg/kg) 0.5 h before i.pl. carrageenan injection (200 μg/cavity), and 0.5 h (panel A) or 2 h (panel B) after carrageenan injection, the pleural cavities of mice were washed, the cells were removed by centrifugation, and the supernatants were used for the determination of KC with a commercially available ELISA. CMC + PBS indicates the carboxymethylcellulose/PBS-treated group. The results are expressed as the mean ± S.E.M. of 3 mice/group; *$P<0.001$; **$P<0.01$.

There are several experimental studies that support an anti-inflammatory role for nicotinamide and other vitamin B components. Most of this evidence has been based on the shared ability of these molecules to inhibit PARP-1, impairing the transcription of pro-inflammatory genes and down-regulating pathways of inflammation and tissue injury (Sodhi et al., 2010), and reduce NF-κB activation (Crowley et al., 2000; Grange et al., 2009; Pero et al., 1999), decreasing the production of the inflammatory cytokines IL-1β, TNF-α (Fukuzawa et al., 1997), IL-6, and IL-8 (Ungerstedt et al., 2003) and lipid mediators such as PGE_2 (Sanchez-Fidalgo et al., 2007). Moreover, PARP-1-deficient mice exhibit decreased transcription and expression of IL-1β, monocyte chemotactic protein (MCP)-1, and TNF-α (Ba and Garg, 2011).

However, to date, a pharmacological approach for the treatment of inflammatory diseases based on nicotinamide administration has not been developed, and the mechanisms involved in the anti-inflammatory activities of nicotinamide are not yet fully understood. Moreover, the effects of nicotinamide on the recruitment of leukocytes require further clarification. In the present study, we investigated the ability of nicotinamide to inhibit neutrophil recruitment in the pleural cavity of mice in response to different inflammatory stimuli. We focused on the ability of nicotinamide to inhibit neutrophil migration in carrageenan-induced pleurisy in mice and the rolling and adhesion of neutrophils on the vessel wall in response to carrageenan.

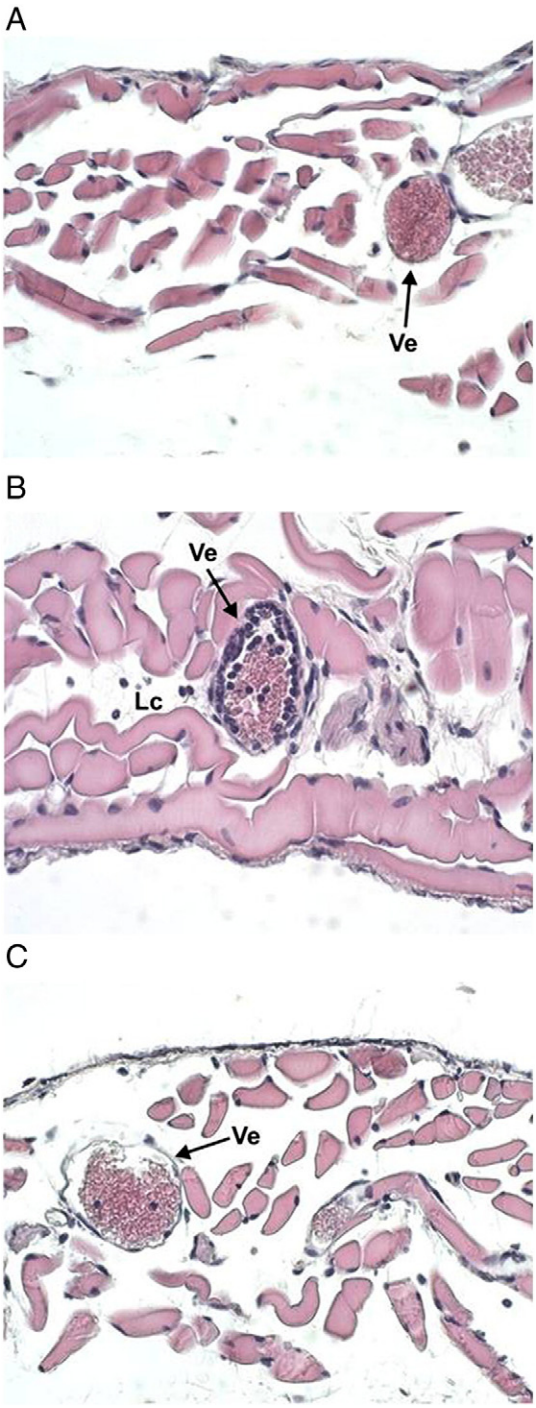

Fig. 4. Histology of the microvasculature in cremaster muscles obtained from double p.o. nicotinamide-treated mice.Mice were treated p.o. with nicotinamide (1000 mg/kg) 0.5 h before and 1 h after the injection of carrageenan (100 μg/0.1 ml) in the left side of the scrotal tissues. The cremaster muscles were removed 4 h after carrageenan injection and fixed in 10% neutral buffered formalin. The tissues were dehydrated in an ethanol series, embedded in paraffin, cut into 4-μm sections, stained with hematoxylin and eosin and examined under direct light microscopy (200×). Panels A, B, and C represent the histological preparations obtained from carboxymethylcellulose + PBS-treated mice, carboxymethylcellulose + carrageenan-treated mice, or nicotinamide + carrageenan-treated mice, respectively. Ve = vessel and Lc = leukocyte.

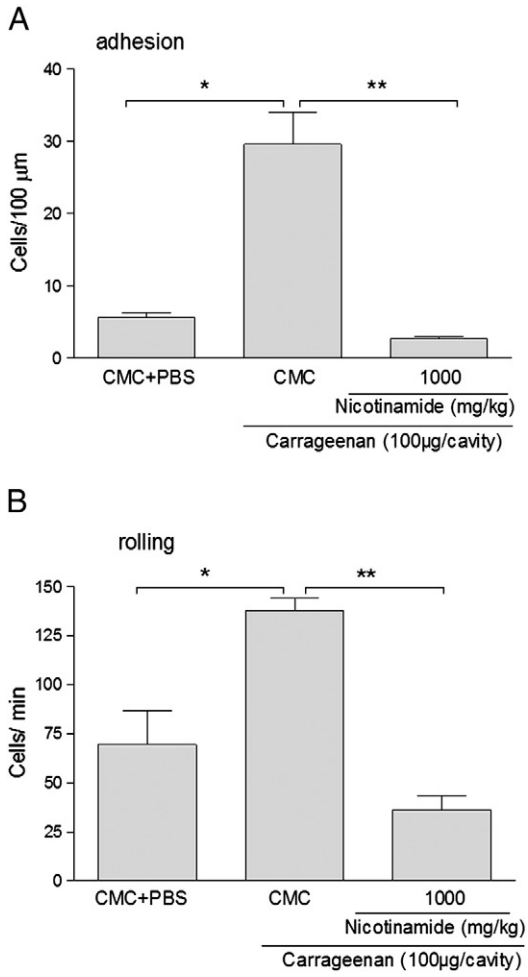

Fig. 5. Nicotinamide treatment reduces the number of rolling and adhesion neutrophils on the vessel wall of mouse cremaster muscles. Mice were treated p.o. with nicotinamide (1000 mg/kg) 0.5 h before and 1 h after the injection of carrageenan (100 μg/0.1 ml) in the left side of the scrotal tissues. The number of adherent (A) and rolling (B) leukocytes was determined using fluorescence microscopy 4 h after carrageenan injection. CMC + PBS indicates the carboxymethylcellulose/PBS-treated group. The results are expressed as the mean ± S.E.M. of 3–4 mice/group; *P < 0.05; **P < 0.001.

The initial experiments were designed to investigate the ability of nicotinamide to inhibit carrageenan- or zymosan-induced neutrophil recruitment in a mouse pleurisy model. The doses of nicotinamide used were based on previous studies with nicotinic acid (Brown et al., 1981; Wang et al., 1990), and the treatment protocol utilized was based on a previous study by Nagai et al. (Nagai et al., 1994). In the present study, nicotinamide effectively inhibited carrageenan-induced neutrophil migration when administered twice, i.e., 30 min before and 1 h after carrageenan challenge. However, a single administration of nicotinamide at 2 h after carrageenan challenge failed to inhibit carrageenan-induced neutrophil recruitment into the mouse pleural cavity, suggesting that nicotinamide is ineffective in inhibiting neutrophil recruitment when the inflammatory process has been established. These results are in accordance with previous studies in a bleomycin-induced inflammation model in hamsters, in which nicotinic acid treatment reduced bleomycin-induced neutrophil accumulation in the lungs. Interestingly, in these experiments, nicotinic acid treatment significantly decreased bleomycin-induced lung PARP activity (Brown et al., 1981). Indeed, PARP treatment with nicotinamide or with PARP inhibitor 3-aminobenzamide (3-AB) inhibited neutrophil infiltration in the paws of rats in zymosan-activated plasma-induced paw edema (Cuzzocrea et al., 1999a) and leukocyte infiltration in carrageenan-induced pleurisy in rats (Cuzzocrea et al., 1999b).

Carrageenan is a well-known inflammatory stimulus that is utilized in *in vivo* studies of neutrophil migration and elicits the production of inflammatory mediators, including the chemoattractants molecules LTB_4 and IL-8/KC, which in turn contribute to the development and

progression of the inflammatory response (Saleh et al., 1999). Nicotinamide was effective in inhibiting the production of KC, when measured 30 min after carrageenan injection.

Because LTB_4 and IL-8/KC mediate murine neutrophil recruitment *via* the activation of receptors present on the surface of neutrophils (Okuno et al., 2005; Yokomizo et al., 1997) and carrageenan has been shown to stimulate tissue cells and leukocytes to release these chemoattractant molecules (Saleh et al., 1999), experiments were performed to investigate whether nicotinamide was also effective in inhibiting neutrophil recruitment in response to these chemoattractants. Nicotinamide treatment inhibited neutrophil recruitment induced by i.pl. injection of LTB_4 or IL-8. These results demonstrate the ability of nicotinamide to inhibit the action of these chemotactic molecules that are released in turn at the inflammatory site during early inflammation, suggesting that the effects of nicotinamide on neutrophil recruitment may be due to the control of early events related to neutrophil migration, such as the impairment of neutrophil chemoattractant molecules.

Interestingly, nicotinamide appears to be more potent in inhibiting zymosan-induced neutrophil recruitment than carrageenan-induced pleurisy because lower doses of nicotinamide were required to inhibit neutrophil recruitment in response to zymosan. Zymosan leads to the activation of the complement system cascade and the subsequent production of anaphylatoxins, chemoattractants to neutrophils (Camussi et al., 1980). Thus, the inhibition of the complement system may be involved in the inhibitory effects of nicotinamide on zymosan-induced neutrophil recruitment, supporting an additional role for nicotinamide action during the initial phase of events that promote leukocyte migration from the blood vessels into the tissues.

We have demonstrated that nicotinamide treatment abolished the rolling and adherence of neutrophils, thereby impairing the ability of neutrophils to migrate from blood vessels into tissues. Our intravital results are in agreement with studies by Von Lukowicz et al. (2008) in which the pharmacological inhibition of PARP-1 enzymatic activity and the genetic deletion of PARP-1 reduced the expression of vascular cell adhesion molecule (VCAM)-1 and P- and E-selectin (von Lukowicz et al., 2008). We speculate that in the carrageenan model, nicotinamide may act through the down-regulation of the expression of adhesion molecules necessary for the induction of neutrophil efflux from blood vessels into the tissues; however, additional studies are needed to confirm this hypothesis.

5. Conclusion

In conclusion, we demonstrate a key role for nicotinamide in inhibiting neutrophil recruitment in a mouse pleurisy model. Taken together, our results strongly indicate that the effects of nicotinamide in inhibiting neutrophil recruitment in carrageenan-induced pleurisy may be explained by their ability to inhibit the action of IL-8 and LTB_4, decrease KC production, and impair early events that regulate the adhesion and rolling of leukocytes in the microcirculation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejphar.2012.04.014.

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